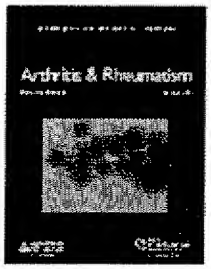
EXHIBIT A



Arthritis & Rheumatism

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Volume 50, Issue 12 , Pages 3994 - 4001

Published Online: 8 Dec 2004

Research Article

Efficacy and tolerability of a selective α_{2C}-adrenergic receptor blocker in recovery from cold-induced vasospasm in scleroderma patients: A single-center, double-blind, placebo-controlled, randomized crossover study

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Funded by:
- Otsuka Maryland Research Institute, Inc.

ABSTRACT

Objective

OPC-28326 is a selective α-adrenergic antagonist with preferential binding to the α_{2C}-adrenergic receptor (α_{2C}-AR) subtype. This study observed the effect of OPC-28326 on skin temperature and digital blood flow following an acute cold challenge in patients with Raynaud's phenomenon secondary to scleroderma.

Methods

The study was designed as a single-center, double-blind, placebo-controlled, randomized, 3-period crossover study of OPC-28326 (oral doses of 10 mg or 40 mg) or placebo. The primary outcome measures were the time to recover 50% and 70% of the fall (induced by cold challenge) in baseline digital skin temperature.

Results

Twelve of 13 enrolled patients completed the study. The mean time to achieve 50% and 70% recovery of the change in prechallenge digital skin temperature was shorter after the OPC-

28326 40-mg dose than after placebo (50% recovery at 5.8 minutes versus 10.0 minutes [P = 0.02]; 70% recovery at 13.8 minutes versus 19.5 minutes [P = 0.01]). These recovery times tended to be shorter in the 10 mg OPC-28326 group as well, but the difference versus placebo was not significant (50% recovery at 9.0 minutes versus 10.0 minutes [P = 0.65]; 70% recovery at 15.3 minutes versus 19.5 minutes [P = 0.07]). Total digital blood flow tended to be lower prior to the cold challenge and after administration of 40 mg OPC-28326, as compared with that after placebo, but the difference was not significant. Symptoms that were potentially drug-related were reported more frequently with 40 mg OPC-28326 than with 10 mg OPC-28326 or with placebo, but none were serious or sustained.

Conclusion

OPC-28326 at doses of 10 mg and 40 mg was well tolerated during this study. The shorter time to skin temperature recovery after 40 mg OPC-28326 suggests that selective α_{2C}-AR blockade improves digital skin perfusion during recovery from cooling in patients with Raynaud's phenomenon secondary to scleroderma.

Received: 24 March 2004; Accepted: 23 August 2004

DIGITAL OBJECT IDENTIFIER (DOI)

10.1002/art.20665 About DOI

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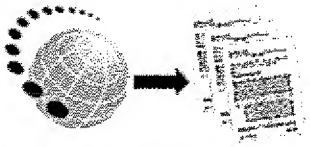

EXHIBIT B

Am J Physiol Regulatory Integrative Comp Physiol 283: R287–R295, 2002;
10.1152/ajpregu.00123.2002.

invited review

Physiological significance of α_2-adrenergic receptor subtype diversity: one receptor is not enough

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Philipp, Melanie, Marc Brede, and Lutz Hein. Physiological significance of α_2-adrenergic receptor subtype diversity: one receptor is not enough. *Am J Physiol Regulatory Integrative Comp Physiol* 283: R287–R295, 2002; 10.1152/ajpregu.00123.2002.—α_2-Adrenergic receptors mediate part of the diverse biological effects of the endogenous catecholamines epinephrine and norepinephrine. Three distinct subtypes of α_2-adrenergic receptors, α_{2A}, α_{2B}, α_{2C}, have been identified from multiple species. Because of the lack of sufficiently subtype-selective ligands, the specific biological functions of these receptor subtypes were largely unknown until recently. Gene-targeted mice carrying deletions in the genes encoding for individual α_2-receptor subtypes have added important new insight into the physiological significance of adrenergic receptor diversity. Two different strategies have emerged to regulate adrenergic signal transduction. Some biological functions are controlled by two counteracting α_2-receptor subtypes, e.g., α_{2A}-receptors decrease sympathetic outflow and blood pressure, whereas the α_{2B}-subtype increases blood pressure. Other biological functions are regulated by synergistic α_2-receptor subtypes. The inhibitory presynaptic feedback loop that tightly regulates neurotransmitter release from adrenergic nerves also requires two receptor subtypes, α_{2A} and α_{2C}. Similarly, nociception is controlled at several levels by one of the three α_2-receptor subtypes. Further investigation of the specific function of α_2-subtypes will greatly enhance our understanding of the relevance of closely related receptor proteins and point out novel therapeutic strategies for subtype-selective drug development.

adrenergic receptors; transgenic mice; gene targeting

ADRENERGIC RECEPTORS FORM the interface between the endogenous catecholamines epinephrine and norepinephrine and a wide array of target cells in the body to mediate the biological effects of the sympathetic nervous system. To date, nine distinct adrenergic receptor subtypes have been cloned from several species: α_{1A}, α_{1B}, α_{1D}, α_{2A}, α_{2B}, α_{2C}, β_1, β_2, and β_3 (11). For many of these receptors, their precise physiological functions and their therapeutic potential have not been fully elucidated. Only for β-adrenergic receptors have sufficiently subtype-selective ligands been developed that have helped to identify the physiological significance of β_1-, β_2-, and β_3-receptors, some of which have entered clinical medicine. Selective agonists for the β_2-adrenergic receptor play an important role in asthma therapy, whereas β_1-receptor antagonists are first-line medication for patients with hypertension, coronary heart disease, or chronic heart failure (8, 20, 50). For α_1-receptors, subtype-selective ligands that can diminish the symptoms of benign prostate hyperplasia without causing hypotension have just entered clinical therapy (33). Despite the fact that α_2-adrenergic receptors serve a number of physiological functions in vivo and have great therapeutic potential, no sufficiently subtype-selective ligands are clinically available yet. Despite this fact, non-subtype-selective α_2-receptor agonists like clonidine, medetomidine, and brimonidine are being used to treat patients with hypertension, glaucoma, tumor pain, postoperative pain, and shivering or

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to block the symptoms of sympathetic overactivity during drug withdrawal (66). Unfortunately, the fields of therapeutic application and unwanted side effects are overlapping, e.g., α_2-receptor-mediated sedation is an important problem for treatment of hypertension. Severe side effects are one reason why α_2-receptor agonists are only second-line antihypertensive agents. It is tempting to speculate that α_2-receptor-mediated therapy could be greatly improved and advanced if receptor subtype-selective ligands were available. However, before developing specific ligands, the therapeutic targets have to be identified. Recently, transgenic and gene-targeted mouse models have added considerable information about individual adrenergic receptor subtypes (15, 25, 37, 39, 53, 54). This review focuses on the specific functions of the three α_2-adrenergic receptor subtypes in mouse models carrying targeted deletions in the genes encoding for α_2-receptors.

α_2-ADRENERGIC RECEPTOR GENES

So far, three distinct genes have been identified from several species that encode for separate subtypes of α_2-adrenergic receptors (11). From these genes, three α_2-receptors are synthesized, termed α_{2A}, α_{2B}, and α_{2C}. The pharmacological ligand binding characteristics of the α_{2A}-subtype differ significantly between different species, thus giving rise to the pharmacological subtypes α_{2A} in humans, rabbits, and pigs and α_{2D} in rats, mice, and guinea pig (11). This species variation is at least in part due to a single amino acid variation in the fifth transmembrane domain of the α_{2A}-receptor that renders this receptor less sensitive to yohimbine binding (34).

GENE-TARGETED MICE LACKING INDIVIDUAL α_2-RECEPTOR SUBTYPES

Several mouse lines have been established by gene targeting that do not express functional α_2-adrenergic receptors (2, 35, 36). All of these mice developed apparently normally, although mice lacking α_{2B}-adrenergic receptors were not born at the expected Mendelian ratios, indicating that this receptor may play a role during embryonic development (13, 35).

In addition, a point mutation has been introduced into the α_{2A}-receptor gene (α_2-D79N) to evaluate the physiological role of separate intracellular signaling pathways of this receptor in vivo (38). The D79N mutation substitutes asparagine for an aspartate residue at position 79, which is predicted to lie within the second transmembrane region of the α_{2A}-receptor and is highly conserved among G protein-coupled receptors. In vitro, the α_{2A}-D79N receptor has been shown to be deficient in coupling to K^+ channel activation (76). However, in vivo this point mutation was found to be deficient in K^+ current activation and Ca^{2+} channel inhibition (32). Surprisingly, the density of α_{2A}-D79N receptors in the mouse brain was decreased to ~20% of the normal level (38). Thus, in most (but not all) functional tests, the α_{2A}-D79N receptor had characteristics resembling a functional "knockout" of the α_{2A}-receptor (40). One important exception was the observation that the presynaptic inhibitory function of the α_{2A}-D79N receptor was normal or only slightly blunted in intact tissues (2). Most likely, the decreased expression of α_{2A}-D79N receptors in vivo rather than a selective defect in receptor signaling seems to be important for the "functional knockout." At the presynaptic side, a high number of spare receptors is characteristic for α_2-receptor function, i.e., activation of very few α_2-receptors results in maximal presynaptic inhibition of transmitter release (1). Thus the reduced number of presynaptic α_{2A}-D79N receptors may still be sufficient for presynaptic control, whereas the decreased receptor density may compromise receptor signal transduction at other sites with a smaller receptor reserve.

WHICH α_2-RECEPTOR SUBTYPE IS THE PRESYNAPTIC REGULATOR?

α_2-Adrenergic receptors were initially characterized as presynaptic receptors that serve as parts of a negative feedback loop to regulate the release of norepinephrine (71). Soon it was shown that α_2-receptors are not restricted to presynaptic locations but also have postsynaptic functions (Fig. 1*A*). With the use of an array of pharmacological antagonists, the α_{2A}-receptor was predicted to be the major inhibitory presynaptic receptor regulating release of norepinephrine from sympathetic neurons as part of a feedback loop (82). However, in some tissues, the α_{2C}-receptors were considered to be in the inhibitory presynaptic receptor (55).

With the genetic deletion of individual α_2-receptor genes in mice, this classification of the presynaptic autoreceptor subtype was challenged. In mice lacking the α_{2A}-subtype, presynaptic feedback regulation was severely impaired but not abolished, indicating that indeed the α_{2A}-receptor is the major autoreceptor in sympathetic neurons (Fig. 1*A*) (2, 26). Most surprisingly, the α_{2C}-receptor turned out to function as an additional presynaptic regulator in all central and peripheral nervous tissues investigated (Fig. 1*A*) (2, 9, 26, 70, 79, 80). However, the relative contributions of α_{2A}- and α_{2C}-receptors differed between central and peripheral nerves, with the α_{2C}-receptor being more prominent in sympathetic nerve endings than in central adrenergic neurons. α_{2A}- and α_{2C}-receptors differ in their time course of expression after birth (65). While α_{2A}-mediated autoinhibition of neurotransmitter release is already operative immediately after birth, the α_{2C}-receptor function is established later in mice (65).

Furthermore, the α_2-autoreceptor subtypes could be distinguished functionally: α_{2A}-receptors inhibited transmitter release significantly faster and at higher action potential frequencies than the α_{2C}-receptors (Fig. 1*B*) (9, 26, 62). When α_{2A}- and α_{2C}-receptors were stably expressed together with N-type Ca^{2+} channels or with G protein-coupled inwardly rectifying K^+ (GIRK) channels, no differences in the activation kinetics of these two receptor subtypes were detected at identical levels of receptor expression (10). However,

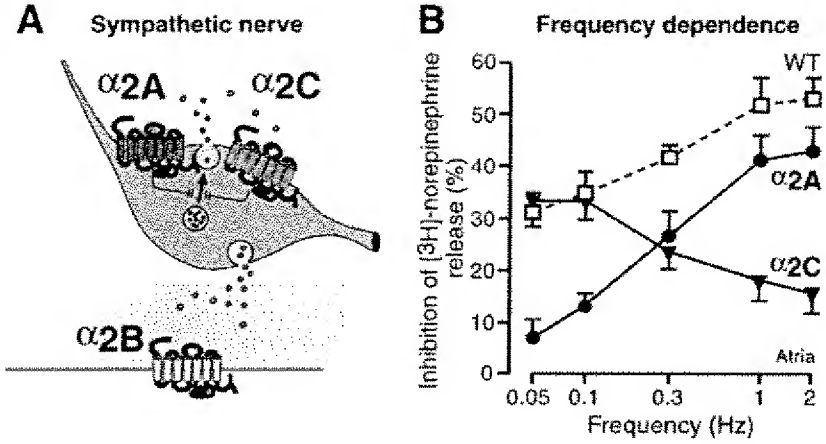

Fig. 1. Presynaptic α_2-adrenergic receptor subtypes. *A*: in sympathetic or central adrenergic nerves, α_{2A}- and α_{2C}-receptors operate as inhibitory autoreceptors to control neurotransmitter release. α_{2B}-Receptors are located on postsynaptic cells to mediate the effects of catecholamines released from sympathetic nerves, e.g., vasoconstriction. *B*: presynaptic α_{2A}- and α_{2C}-receptors can be distinguished functionally. In intact tissue slices from mouse heart atria, α_{2A}-receptors inhibit norepinephrine release from sympathetic nerves primarily at high stimulation frequencies, whereas the α_{2C}-receptor can also operate at very low frequencies to control basal norepinephrine release. WT, wild type. Data adapted from Ref. 26.

when receptor GIRK channel deactivation after removal of norepinephrine was followed, the α_{2C}-receptor was found to be active for a significantly longer time than the α_{2A}-subtype irrespective of the level of receptor expression. This difference in α_2-receptor deactivation kinetics could be explained by the higher affinity of norepinephrine for the α_{2C}- than for the α_{2A}-receptor subtype (10). This property makes the α_{2C}-receptor particularly suited to control neurotransmitter release at low action potential frequencies (Fig. 1) (26). In contrast, the α_{2A}-receptor seems to operate primarily at high stimulation frequencies in sympathetic nerves and may thus be responsible for controlling norepinephrine release during maximal sympathetic activation.

α_2-Adrenergic receptors not only inhibit release of their own neurotransmitters (autoreceptors) but can also regulate the exocytosis of a number of other neurotransmitters in the central and peripheral nervous system. In the brain, α_{2A}- and α_{2C}-receptors can inhibit dopamine release in basal ganglia (9) as well as serotonin secretion in mouse hippocampal or brain cortex slices (61). In contrast, the inhibitory effect of α_2-agonists on gastrointestinal motility was mediated solely by the α_{2A}-subtype (63).

Part of the functional differences between α_{2A}- and α_{2C}-receptors may be explained by their distinct subcellular localization patterns (Fig. 2) (14, 47, 86, 87). In cultured sympathetic neurons from newborn mice, functional presynaptic α_2-receptors develop to inhibit voltage-dependent Ca^{2+} channels and norepinephrine release (77, 78). In sympathetic neurons, only the α_{2A}-subtype but not the α_{2C}-receptor contributed to inhibition of neurotransmitter release (81). Remarkably, in-

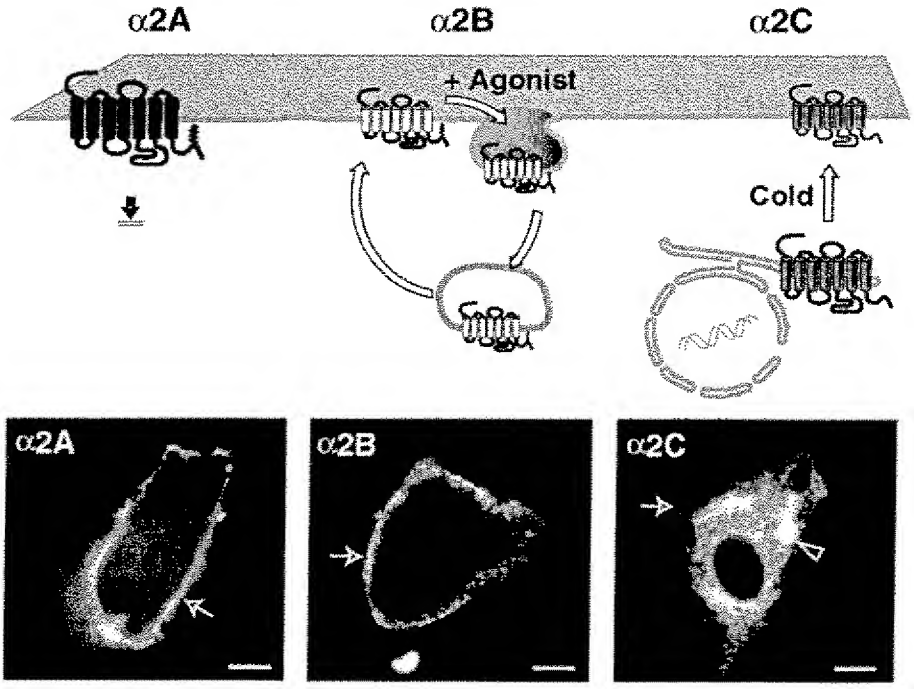

Fig. 2. α_2-Adrenergic receptors differ in their trafficking itineraries in cells. When expressed in rat1 fibroblasts, α_{2A}- and α_{2B}-receptors are targeted to the plasma membrane (immunofluorescence images). On stimulation with agonist, only α_{2B}-receptors are reversibly internalized into endosomes. α_{2C}-Receptors are primarily localized in an intracellular membrane compartment, from where the α_{2C}-receptors can be translocated to the cell surface after exposure to cold temperature (29). *Bottom*: murine α_2-receptor subtypes after transient transfection into rat1 fibroblasts as described previously (14). Arrows point to α_2-receptors residing in the plasma membrane; arrowhead marks α_{2C}-receptors in an intracellular compartment.

hibition of Ca^{2+} channels located on neuronal cell bodies and dendrites was mediated by both α_{2A}- and α_{2C}-receptors. Thus α_{2C}-receptors in neurons may require a specific itinerary to guide their expression to axonal termini.

BLOOD PRESSURE REGULATION

α_2-Receptors are involved in the control of blood pressure homeostasis at a number of locations (Fig. 3). Nonselective activation of α_2-receptors usually leads to a biphasic blood pressure response: after a short hypertensive phase that is more pronounced after rapid intravenous injection, arterial pressure falls below the baseline. After oral application of α_2-agonists, the hypotensive action prevails and is being used to treat elevated blood pressure in hypertensive patients. Interestingly, the two phases of the pressure response are mediated by two different α_2-receptor subtypes in vivo: α_{2B}-receptors are responsible for the initial hypertensive phase, whereas the long-lasting hypotension is mediated by α_{2A}-receptors (2, 35, 38). Thus the α_{2A}-receptor is a therapeutic target for subtype-selective antihypertensive agents. The blockade of α_2-receptors may be of therapeutic benefit in patients with atherosclerotic coronary arteries (3), whereas it is still unknown which α_2-receptor subtype is responsible for the vasoconstriction in humans. An insertion/deletion polymorphism with decreased receptor desensitization of the α_{2B}-receptor subtype is associated with an increased risk for acute coronary events (69).

Some evidence indicates that α_{2A}-receptors also participate to a smaller degree in the vasoconstrictor action of α_2-agonists in mice (38). Bolus injection of norepinephrine caused transient hypertension in wild-type mice and in α_{2B}- and α_{2C}-deficient mice but not in mice lacking the α_{2A}-receptor (16). Vascular α_2-receptor subtypes may be differentially distributed between vascular beds. When α_2-agonists were injected into the carotid artery, most of the hypertensive response to α_2-activation was mediated by the α_{2B}-receptor (35), whereas injection into the femoral artery showed a blunted hypertensive effect in mice with the α_{2A}-D79N receptor (38). In some arteries, α_2-mediated vasoconstriction may even predominate over α_1-receptor-induced contraction, and decreased α-receptor responsiveness may contribute to elevated blood flood in tissue inflammation, e.g., arthritis (45).

In addition to its role as a vasoconstrictor, the α_{2B}-receptor seems to be required for the development of salt-sensitive hypertension (Fig. 3) (22, 41–43). Nephrectomy followed by Na^+ loading has been established as a model of hypertension in mice (22). In this system, the development of hypertension depends on increased vasopressin release and sympathetic activation (21). Bilateral nephrectomy and saline infusion raised blood pressure in wild-type and in α_{2A}- and α_{2C}-receptor-deficient mice. However, in α_{2B}-deficient animals a small fall in arterial pressure was observed (41). Recent experiments with α_{2B}-antisense oligonucleotide injection into the lateral brain ventricle suggest that a central α_{2B}-adrenergic receptor is necessary for induction of salt-dependent hypertension (31).

Under certain conditions, even the α_{2C}-receptor subtype may contribute to vascular regulation: when kept below 37°C for a while, cutaneous arteries of the mouse tail show an α_{2C}-receptor-dependent vasoconstriction that could not be observed when the vessel segments were incubated at body temperature (12). This finding may be of great therapeutic interest for the treatment of Raynaud's disease. Patients with Raynaud's phenomenon suffer from severe periods of vasoconstriction of their fingers and toes that are usually triggered by exposure to cold. Treatment of these patients with α_2-adrenergic antagonists diminished the vasoconstriction (19). Interestingly, silent α_{2C}-receptors may be translocated from an intracellular receptor pool to the cell surface on cooling (Fig. 2) (29). This phenomenon has been observed in human embryonic kidney (HEK-293) cells transfected with recombinant α_{2C}-receptors: cooling of cells to 28°C evoked a redistribution of α_{2C}-receptors from the Golgi apparatus to the plasma membrane within 1 h (29). Thus inhibition of α_{2C}-receptors may prove an effective treatment for Raynaud's phenomenon.

In addition to these vascular and central neuronal mechanisms, renal α_2-receptors may be involved in the

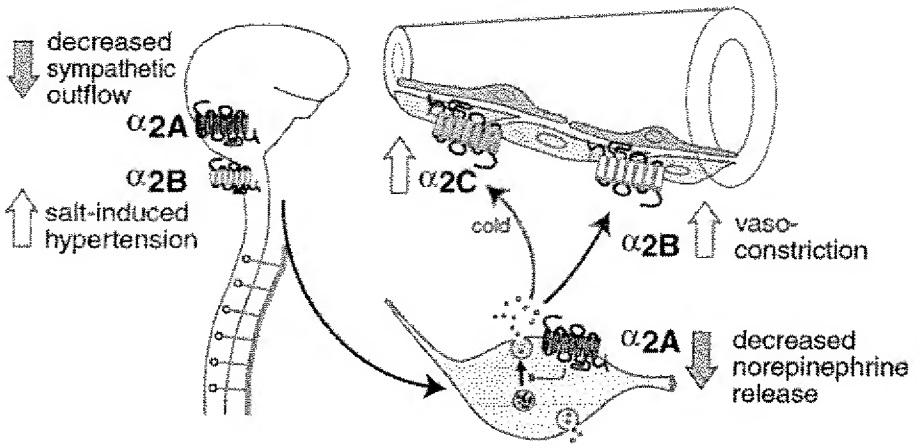

Fig. 3. Integrative regulation of blood pressure by different α_2-adrenergic receptor subtypes. Activation of α_{2A}-receptors leads to a decrease in blood pressure by inhibiting central sympathetic outflow as well as norepinephrine release from sympathetic nerves (2, 38). α_{2B}-Receptors may counteract this effect by causing direct vasoconstriction and salt-induced hypertension (22, 35). α_{2C}-Receptors participate in α_2-mediated vasoconstriction after exposure to cold temperature (12).

long-term regulation of blood pressure and fluid and electrolyte homeostasis (48, 49). Activation of renal vascular α_{2B}-receptors may lead to an increase in medullary NO production and thus counteract the vasoconstrictor effects of norepinephrine in the renal medulla (90). Via this mechanism, α_{2B}-receptors may be essential in the regulation of renal medullary blood flow and oxygen supply.

ANALGESIA

α_2-Agonists are potent analgesics, and they can potentiate the analgesic effect of opioids (75, 85, 88). Recent data indicate that all three α_2-receptor subtypes are involved in the regulation of pain perception in the mouse (Fig. 4).

The α_{2A}-receptor mediates the antinociception induced by systemically applied α_2-agonists, including clonidine and dexmedetomidine (18, 74). Compared with control mice, α_2-agonists were completely ineffective as an antinociceptive agent in the tail immersion or substance P test in α_{2A}-D79N mice (27). The α_{2A}-D79N mutation also blocked the synergy seen in wild-type mice between α_2-agonists and delta-opioid agonists (74). Interestingly, α_{2A}-receptor-deficient mice showed a reduced antinociceptive effect to isoflurane (30). However, not all α_2-receptor agonists required functional α_{2A}-receptors for their antinociceptive effect (Fig. 4). The imidazoline/α_2-receptor ligand moxonidine caused spinal antinociception that was at least partially dependent on α_{2C}-receptors (17).

Surprisingly, nitrous oxide, which is used as a potent inhalative analgesic during anesthesia, requires the α_{2B}-subtype for its antinociceptive effect (Fig. 4) (23, 60). Supraspinal opioid receptors and spinal α_{2B}-receptors are involved in the analgesic pathway for nitrous oxide. Activation of endorphin release in the periaqueductal gray by nitrous oxide stimulates a descending noradrenergic pathway that releases norepinephrine onto α_{2B}-receptors in the dorsal horn of the spinal cord (89). In mice lacking α_{2B}-receptors, the analgesic effect of nitrous oxide was completely abolished (60).

SEDATION

α_2-Agonists are used in the postoperative phase or in intensive care as sedative, hypnotic, and analgesic agents (44, 66). The sedative effects of α_2-agonists in mice are solely mediated by the α_{2A}-receptor subtype (32). α_{2A}-D79N mice showed no sedative response to the α_2-agonist dexmedetomidine (32). In contrast, mice lacking the α_{2B}- or α_{2C}-receptors did not differ in their sedative response from wild-type control mice (27, 59). Similarly, the anesthetic-sparing effect of α_2-agonists was completely abolished in α_{2A}-D79N mice (32).

The hypnotic effect of α_2-agonists is most likely mediated in the locus ceruleus. Neurons of the locus ceruleus express α_{2A}-adrenergic receptors at very high density (84). Furthermore, α_{2A}-antisense oligonucleotide injection into the locus ceruleus in rats attenuated the sedative effects of exogenous α_2-agonists (46).

BEHAVIOR

Because of their widespread distribution in the central nervous system, α_2-receptors affect a number of behavioral functions (5, 56, 57, 67). In particular, the α_{2C}-receptor subtype has been demonstrated to inhibit the processing of sensory information in the central nervous system of the mouse (for a recent review, see Ref. 64). Activation of α_2-receptors also resulted in locomotor inhibition. While direct activation of α_2-receptors by dexmedetomidine did not alter spontaneous motor activity in α_{2C}-receptor-deficient mice (59), D-amphetamine stimulated locomotor activity to a greater extent in α_{2C}-deficient mice than in wild-type mice (58).

Mice overexpressing α_{2C}-receptors were impaired in spatial and nonspatial water maze tests, and an α_2-

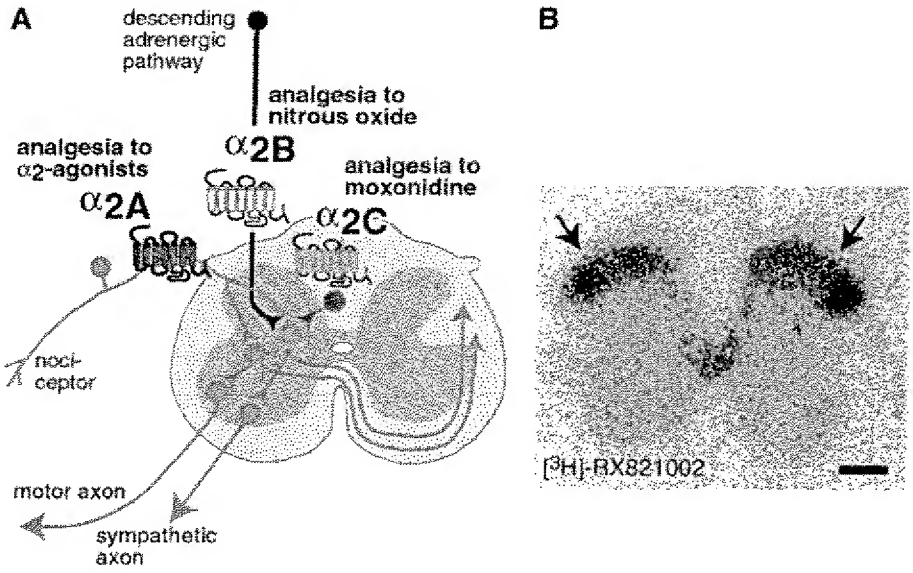

Fig. 4. Three α_2-adrenergic receptor subtypes are involved in the control of pain perception in mice. *A*: schematic representation of α_2-receptor subtypes controlling spinal nociception. *B*: distribution of α_2-receptors in the mouse spinal cord by autoradiography with a non-subtype-selective α_2-receptor antagonist (9). In the spinal cord, the highest density of α_2-adrenergic receptors was observed in the superficial layers of the dorsal horns (*B*, arrows). Here, all 3 α_2-receptor subtypes control incoming nociceptive impulses: α_{2A}-receptors are required for the analgesic effect of systemically applied α_2-agonists, spinal α_{2C}-receptors contribute to the moxonidine-mediated analgesia, and α_{2B}-receptors are required for the spinal antinociceptive effect of nitrous oxide. See text for references. The autoradiogram shown in *B* was kindly provided by K. Hadamek, Würzburg, Germany.

antagonist fully reversed the water maze escape defect in these mice (4–6). The α_2-agonist dexmedetomidine increased swimming distance more effectively in wild-type mice than in α_{2C}-receptor-deficient mice (4). Activation of α_{2C}-receptors disrupts execution of spatial and nonspatial search patterns, whereas stimulation of α_{2A}- and/or α_{2B}-receptors may actually improve spatial working memory in mice (7). It may be concluded that novel agonists devoid of α_{2C}-receptor affinity can modulate cognition more favorably than non-subtype-selective drugs.

Altered startle reactivity and attenuation of the inhibition of the startle reflex by an acoustic prepulse have been observed in schizophrenia, and disrupted prepulse inhibition has frequently been used as an animal model for drug antipsychotic drug development. Interestingly, α_{2C}-receptor-deficient mice had enhanced startle responses, diminished prepulse inhibition, and shortened attack latency in the isolation-aggression test (57). Thus drugs acting via the α_{2C}-receptor may have therapeutic value in disorders associated with enhanced startle responses and sensorimotor gating deficits, such as schizophrenia, attention deficit disorder, posttraumatic stress disorder, and drug withdrawal. In addition to the α_{2C}-subtype, the α_{2A}-receptor has an important role in modulating behavioral functions. Experiments using gene-targeted mice indicate that the α_{2A}-receptor may play a protective role in some forms of depression and anxiety, and this receptor may mediate part of the antidepressant effects of imipramine (67). Thus α_{2A}- and α_{2C}-receptors complement each other to integrate central nervous system function and behavior.

OTHER PHYSIOLOGICAL FUNCTIONS AND PHARMACOLOGICAL TARGETS

α_2-Receptors are involved in the regulation of body temperature as well as seizure threshold. Activation of central α_{2A}-receptors causes a powerful antiepileptogenic effect in mice (28). Two receptor subtypes, α_{2A} and α_{2C}, may be involved in the hypothermic action of α_2-agonists (27, 59). Another important function of α_2-agonists is their inhibitory effect on intraocular pressure. The α_2-agonists apraclonidine and brimonidine are currently being used to lower intraocular pressure in patients with glaucoma (52, 68). In adipose tissue, α_2-receptors inhibit lipolysis (72, 73) and α_2-receptors are potential targets for the treatment of obesity. Mice expressing human α_2-receptors in fat tissue in vivo, in the absence of β_3-adrenergic receptors, developed high-fat diet-induced obesity (83). However, the precise role of individual α_2-receptor subtypes in the control of lipolysis is unknown at present.

CONCLUSIONS

Genetic deletion of α_2-adrenergic receptor subtype genes in mice has greatly enhanced our understanding of the physiological functions and therapeutic potential of individual α_2-receptor subtypes. α_2-Adrenergic receptors are important regulators of sympathetic tone, neurotransmitter release, blood pressure, and intraocular pressure. α_2-Receptor activation causes sedation and potent analgesia. Further potential therapeutic functions may be unraveled with the help of mouse models with deleted α_2-receptor genes. Before these genetic animal models were available, it was hypothesized that each biological function of α_2-receptors would be mediated by one receptor subtype. Thus it was reasonable to assume that novel subtype-specific pharmacological agonists or antagonists would be of great therapeutic value because of their reduced potential for α_2-receptor-mediated side effects. However, with more and more studies of the α_2-receptor physiology in gene-targeted mice being published, the situation became more complicated than initially anticipated. Indeed, only a few biological functions of α_2-receptors were found to be mediated by one single α_2-adrenergic receptor subtype. Examples are the hypotension or sedation caused by α_{2A}-receptor activation.

For other α_2-receptor-mediated functions, two different strategies seem to have emerged to regulate adrenergic signal transduction: some biological functions are controlled by two counteracting α_2-receptor subtypes, e.g., α_{2A}-receptors decrease sympathetic outflow and blood pressure, whereas the α_{2B}-subtype increases blood pressure by direct vasoconstriction. In contrast, the inhibitory presynaptic feedback loop that tightly regulates neurotransmitter release from adrenergic nerves requires two receptor subtypes, α_{2A} and α_{2C}, with similar but complementary effects. Similarly, pain perception is controlled at several levels of by one of the three α_2-receptor subtypes.

The fact that more than one receptor subtype may be involved in regulating one particular physiological function does not limit the therapeutic potential of novel subtype-selective drugs for α_2-adrenergic receptors. However, it emphasizes that knowledge of the spectrum of in vivo biological effects is mandatory before making precise predictions about the in vivo effects of subtype-specific drugs. For treatment of hypertension, a selective α_{2A}-receptor agonist without affinity for the α_{2B}-receptor might be advantageous. As α_{2B}-receptors counteract the hypotensive effect of α_{2A}-receptor activation, a selective α_{2A}-agonist could be given at a lower dose to achieve similar blood pressure lowering with reduced sedative side effects. In addition, a combination of agonistic and antagonistic properties may become desirable, for instance, for antihypertensives, e.g., α_{2A}-agonist and α_{2B}-antagonist. The primary target for α_2-mediated pain modulation would be the α_{2B}-receptor. As illustrated by the potent analgesic effect of nitrous oxide, α_{2B}-receptor activation might be a very promising analgesic strategy. Whether α_{2C}-receptors are equally effective in inhibiting pain pathways in the spinal cord has to be tested in future studies (17). The main advantage of α_{2B}- or α_{2C}-receptor-specific agonists for antinociception would be their lack of sedative side effects compared with nonselective α_2-agonists that also stimulate α_{2A}-receptors. In addition, they would not cause respiratory depression and

addiction, which are two major problems associated with opioid therapy. In anesthesia and intensive care, the availability of pairs of subtype-selective agonists and antagonists might be of great benefit (66). α_{2A}-Receptor-mediated sedation that can be rapidly reversed by a selective α_2-antagonist may be used in future human anesthesia (as it is already being used with non-subtype-selective agonists/antagonists in veterinary anesthesia). Finally, mice lacking all α_2-receptor subtypes will also be essential tools to determine the function of imidazoline receptors and the potential of future imidazoline receptor drugs (24, 51).

Further investigation of the specific function of α_2-subtypes will greatly enhance our understanding of the relevance of closely related receptor proteins and point out novel therapeutic strategies for subtype-selective drug development.

Our work has been supported by the Deutsche Forschungsgemeinschaft.

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